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Microbial recovery of metals from solids

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Microbial recovery of metals from solids

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Abstract

A variety of both lithotrophic and organotrophic microorganisms are known to mediate the mobilization of various elements from solids mostly by the formation of inorganic and organic acids. Under appropriate environmental conditions, metals are solubilized and extracted from metal-rich materials in subsurface ecosystems by the action of bacteria and fungi. In mine tailings or landfills microbial metal leaching represents a potential environmental hazard. However, these microbial activities can be successfully applied in the industry for the recovery of metals from solid materials such as ores or incineration residues. Microbial leaching processes are currently used for the winning of gold and copper from low-grade ores ('bioleaching'). Solid industrial waste materials such as fly ash, sludges, or dust might also be microbially treated to recover metals for the re-use in metal-manufacturing industries. Bioleaching allows the cycling of metals by a process close to natural biogeochemical cycles reducing the demand for resources such as ores, energy, or landfill space.

Keywords: Bioleaching; Biohydrometallurgy; Heavy metal; Fly ash; Waste incineration residue; Renewable resource

Contents

1. Introduction	605
2. Principles of bacterial metal leaching	606
3. Industrial applications and patents	610
4. Solid wastes as metal resource	610
5. Case study: Fly ash from municipal waste incineration	610
6. Perspectives for bioleaching processes	613
Acknowledgements	614
References	615

1. Introduction

In the light of the expected shortage of non-renew-

able resources, increased efforts are absolutely necessary to open new sources of raw materials with the aid of new or improved technologies. A possibility to obtain metals from low-grade mineral resources is offered by the use of microbiological leaching processes [1,2]. Bioleaching processes are based on the

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Table 1

Concentration of selected elements in residues from municipal waste incineration compared to the elemental composition of the earth crust and ores suitable for economic metal recovery [3–5]

Element	Earth crust (%)	Slag (%)	Fly ash (%)	Ore (%) ^a
Cd	0.00015	< 0.001	0.04	0.2 ^b
Cr	0.02	0.04	0.06	30
Cu	0.007	0.16	0.09	0.2
Ni	0.008	0.014	0.014	1.5
Pb	0.0016	0.06	0.8	4
Sn	0.004	< 0.05	0.6	1
Ti	0.44	0.75	1.1	1
Zn	0.008	0.19	2.7	4

^aThe percentage represents the elemental concentration in ores for economic metal recovery by conventional mining techniques.

^bCd is usually associated with zinc ores (zinc sulfide, zinc carbonate). Within these ores, Cd concentration is approximately 0.2%.

ability of microorganisms (bacteria, fungi) to transform solid compounds resulting in soluble and extractable elements which can be recovered. Applying bioleaching processes, it is possible to obtain metals from natural ores and industrial residues which cannot be handled with conventional techniques [2]. However, bioleaching processes have only recently gained importance in a variety of mineral industries, whereas, in contrast, chemical hydrometallurgical leaching under acidic conditions has been described in many publications [2].

Solids from waste incineration such as slag and ash are considered 'artificial ores' (Table 1). It might become possible to recycle the leached and recovered metals and to re-use them as raw materials by metal-manufacturing industries. In addition, the environmental quality of the residues is improved by the process with respect to a subsequent application of these materials for construction purposes. Bioleaching of waste materials allows the cycling of metals by a process close to natural biogeochemical cycles reducing the demand for resources such as ores, energy, or landfill space. Bioleaching represents a 'clean technology' process with a low cost and low energy level compared with conventional thermal solid waste treatment techniques. Government regulations and research policies that favor 'green' technologies are a key incentive for developing such processes. These find a wide acceptance with the public and in politics. It is a matter of innovative technologies showing a proved market gap.

2. Principles of bacterial metal leaching

Valuable metals have been obtained from ores by leaching for hundreds of years. This process was believed to be a chemical reaction mediated by water and oxygen until 1947, when bacterial catalyzation of iron oxidation and sulfuric acid formation in mine waters was demonstrated [6]. However, the microbial production of sulfuric acid in mine waters had already been suggested 15 years earlier, but attempts to isolate and characterize the strains responsible for the acidification were not successful [6].

A variety of microorganisms are known to catalyze leaching of metals mostly from ore deposits and mine tailings. Above all, autotrophic *Thiobacillus* species as well as heterotrophic *Aspergillus* and *Penicillium* species are described in the literature. Table 2 gives an overview of selected microorganisms known for their bioleaching potential. Excellent detailed reviews of microbial and chemical principles of bioleaching have been published elsewhere [2,10,18,24].

The ability of microorganisms to leach and mobilize metals from solid materials comprises three principles, namely (i) redox reactions, (ii) the formation of organic or inorganic acids, and (iii) the excretion of complexing agents. The mediation by redox reactions is based either on electron transfer from minerals to microorganisms in the case of physical contact between organisms and solids or on bacterial oxidation of Fe^{2+} to Fe^{3+} where ferric iron subsequently catalyzes metal solubilization as an oxidizing agent [25].

Table 2
Selection of microorganisms known to mediate metal bioleaching reactions

Organism	Type	Nutrition	Main leaching agent	pH range	pH optimum	Temperature range	Reference
<i>Acetobacter methanolicus</i>	bacteria	heterotrophic	gluconate	acidiphilic			[7]
<i>Acidimanus brierleyi</i>	bacteria	facult. heterotrophic	sulfuric acid	acidophilic	1.5–3.0	45–75	[8,9]
<i>Acidophilum cryptum</i>	bacteria	heterotrophic	organic acids	2.0–6.0		mesophilic	[10]
<i>Actinonucor</i> sp.	fungi	heterotrophic	oxalate, malate, pyruvate, oxalacetate				[11]
<i>Alternaria</i> sp.	fungi	heterotrophic	oxalate, malate, pyruvate, oxalacetate				[11]
<i>Arthrobacter</i> sp.	bacteria	heterotrophic					[12]
<i>Aspergillus amstelodami</i>	fungi	heterotrophic					[11]
<i>Aspergillus clavatus</i>	fungi	heterotrophic	aspartate				[11]
<i>Aspergillus ficuum</i>	fungi	heterotrophic	oxalate				[13]
<i>Aspergillus fumigatus</i>	fungi	heterotrophic					[11]
<i>Aspergillus niger</i>	fungi	heterotrophic	oxalate, citrate, gluconate, lactate				[5,14]
<i>Aspergillus ochraceus</i>	fungi	heterotrophic	citrate, glutamate				[11]
<i>Bacillus megaterium</i>	bacteria	heterotrophic	citrate, amino acids				[15]
<i>Candida</i> sp.	fungi	heterotrophic					[11]
<i>Cerostanella</i> sp.	fungi	heterotrophic					[11]
<i>Chromobacterium violaceum</i>	bacteria	heterotrophic	cyanide				[9]
<i>Cladosporium resinae</i>	fungi	heterotrophic					[11]
<i>Coriolus versicolor</i>	fungi	heterotrophic					[11]
<i>Corynebacterium</i> sp.	bacteria	heterotrophic					[16]
<i>Crenothrix</i> sp.	bacteria	facult. autotrophic	ferric iron	5.5–6.2		18–24	[10]
<i>Cunninghamiella</i> sp.	fungi	heterotrophic					[11]
<i>Fusarium</i> sp.	fungi	heterotrophic	oxalate, malate, pyruvate, oxalacetate				[11]
<i>Gallionella</i> sp.	bacteria	autotrophic	ferric iron	6.4–6.8		6–25	[10]
<i>Gleophyllum trabecum</i>	fungi	heterotrophic	oxalate				[13]
<i>Leptospirillum ferrooxidans</i>	bacteria	chemolithoautotrophic	ferric iron		2.5–3.0	30	[9,10]
<i>Leptospirillum thermoferrooxidans</i>	bacteria	chemolithoautotrophic	ferric iron		1.7–1.9	45–50	[8]
<i>Leptotrix</i> sp.	bacteria	facult. autotrophic	ferric iron, sulfuric acid	5.8–7.8		5–40	[10]
<i>Metallogenium</i> sp.	bacteria	heterotrophic	ferric iron	3.5–6.8	4.1		[10]
<i>Metallosphaera sedula</i>	bacteria	chemolithoautotrophic	ferric iron, sulfuric acid	acidophilic		extr. thermophilic	[8,10]
<i>Mucor</i> sp.	fungi	heterotrophic	funatate, gluconate				[11]
<i>Paecilomyces variotii</i>	fungi	heterotrophic	malate				[11]
<i>Penicillium brevicompactum</i>	fungi	heterotrophic					[11]
<i>Penicillium cyclopium</i>	fungi	heterotrophic					[11]
<i>Penicillium funiculosum</i>	fungi	heterotrophic					[11]
<i>Penicillium notatum</i>	fungi	heterotrophic	citrate, glutamate				[11]

Table 2 (continued)
Selection of microorganisms known to mediate metal bioleaching reactions

Organism	Type	Nutrition	Main leaching agent	pH range	pH optimum	Temperature range	Reference
<i>Penicillium ochrochloron</i>	fungi	heterotrophic	oxalate				[11]
<i>Penicillium oxalicum</i>	fungi	heterotrophic	oxalate				[13]
<i>Penicillium simplicissimum</i>	fungi	heterotrophic	citrate, oxalate, gluconate				[13]
<i>Penicillium spinulosum</i>	fungi	heterotrophic	oxalate				[11,14]
<i>Penicillium variotii</i>	fungi	heterotrophic					[11]
<i>Phanerochaete chrysosporium</i>	fungi	heterotrophic					[11]
<i>Pichia</i> sp.	fungi	heterotrophic					[11]
<i>Pseudomonas putida</i>	bacteria	heterotrophic	citrate, gluconate				[15]
<i>Rhizopus</i> sp.	fungi	heterophilic	lactate, fumarate, gluconate				[11]
<i>Schizophyllum commune</i>	fungi	heterotrophic	malate				[11]
<i>Sclerotium rolfsii</i>	fungi	heterotrophic	oxalate				[13]
<i>Siderocapsa</i> sp.	bacteria	heterotrophic	ferric iron				[10]
<i>Sulfobacillus thermosulfidooxidans</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid	extremely acidophilic		50	[8]
<i>Sulfobacillus thermosulfidooxidans</i> sub. <i>thermotolerans</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid	extremely acidophilic		37–42	[8]
<i>Sulfobacillus thermosulfidooxidans</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid	extremely acidophilic		50	[8]
sub. <i>asporogenes</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid	acidophilic	2.0–3.0	55–85	[9,10]
<i>Sulfobolus acidocaldarius</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid	0.9–5.8		extremely thermophilic	[8,10]
<i>Sulfobolus ambivalens</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid			extremely thermophilic	[8,10]
<i>Sulfobolus solfataricus</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid			extremely thermophilic	[8,10]
<i>Sulfobolus thermosulfidooxidans</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid			extremely thermophilic	[8,10]
<i>Sulfobolus brierleyi</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid			extremely thermophilic	[17]
<i>Sulfobolus yellowstonii</i>	archaea	chemolithoautotrophic	ferric iron, sulfuric acid			extremely thermophilic	[8]
<i>Sulfurococcus</i> sp.	bacteria	mixotrophic	ferric iron, sulfuric acid	acidophilic		extremely thermophilic	[8]
<i>Thermotrix thiopara</i>	bacteria	chemolithoautotrophic	sulfuric acid	neutral		60–75	[17]
<i>Thiobacillus acidophilus</i>	bacteria	chemolithoautotrophic	sulfuric acid	1.5–6.0	3.0	25–30	[10]
<i>Thiobacillus albertis</i>	bacteria	chemolithoautotrophic	sulfuric acid	2.0–4.5	3.5–4.0	28–30	[10]
<i>Thiobacillus capsulatus</i>	bacteria	chemolithoautotrophic	sulfuric acid				[18]
<i>Thiobacillus concretivorus</i>	bacteria	chemolithoautotrophic	sulfuric acid	0.5–6.0			[10]
<i>Thiobacillus cuprinus</i>	bacteria	chemolithoautotrophic	sulfuric acid				[19]
<i>Thiobacillus delicatus</i>	bacteria	mixotrophic	sulfuric acid		5.0–7.0	25–30	[10]
<i>Thiobacillus denitrificans</i>	bacteria	chemolithoautotrophic	sulfuric acid	5.0–7.0		30	[10]
<i>Thiobacillus ferrooxidans</i>	bacteria	chemolithoautotrophic	ferric iron, sulfuric acid	1.4–6.0	2.4	28–35	[7,9,10]

Table 2 (continued)
Selection of microorganisms known to mediate metal bioleaching reactions

Organism	Type	Nutrition	Main leaching agent	pH range	pH optimum	Temperature range	Reference
<i>Thiobacillus intermedius</i>	bacteria	chemolithoautotrophic	sulfuric acid	1.9–7.0	6.8	30	[10]
<i>Thiobacillus kabobis</i>	bacteria	mixotrophic	sulfuric acid	1.8–6.0	3.0	28	[10]
<i>Thiobacillus neapolitanus</i>	bacteria	chemolithoautotrophic	sulfuric acid	3.0–8.5	6.2–7.0	28	[10]
<i>Thiobacillus novellus</i>	bacteria	chemolithoautotrophic	sulfuric acid	5.0–9.0	7.8–9.0	30	[10]
<i>Thiobacillus organoparus</i>	bacteria	mixotrophic	sulfuric acid	1.5–5.0	2.5–3.0	27–30	[10]
<i>Thiobacillus perometabolis</i>	bacteria	chemolithoheterotrophic	sulfuric acid	2.6–6.8	6.9	30	[10]
<i>Thiobacillus prosperus</i>	bacteria	chemolithoautotrophic	sulfuric acid				[20]
<i>Thiobacillus rubellus</i>	bacteria	chemolithoautotrophic	sulfuric acid		5.0–7.0	25–30	[8,10]
<i>Thiobacillus tepidarius</i>	bacteria	chemolithoautotrophic	sulfuric acid				[21]
<i>Thiobacillus thiooxidans</i>	bacteria	chemolithoautotrophic	sulfuric acid				[22]
<i>Thiobacillus thiooxidans</i>	bacteria	chemolithoautotrophic	sulfuric acid	0.5–6.0	2.0–3.5	10–37	[10]
<i>Thiobacillus thioparus</i>	bacteria	chemolithoautotrophic	sulfuric acid	4.5–10.0	6.6–7.2	11–25	[10]
<i>Thiobacillus versutus</i>	bacteria	chemolithoautotrophic	sulfuric acid		8.0–9.0		[10]
<i>Trametes versicolor</i>	fungi	heterotrophic					[11]
<i>Trichoderma harzianum</i>	bacteria	heterotrophic					[11]
<i>Trichoderma viride</i>	bacteria	heterotrophic					[23]
<i>Yarrowia lipolytica</i>	fungi	heterotrophic	citrate				[11]

3. Industrial applications and patents

Leaching processes have been investigated for the recovery of cadmium, cobalt, copper, gold, manganese, nickel, plutonium, silver, uranium, and zinc from ores and concentrates as well as for the removal of pyrite from coal [26–30]. Currently, these microbiological technologies are used on an industrial scale only for the recovery of copper, gold, or uranium from ores [9,25,30]. Subjecting refractory gold ore to microbial treatment prior to conventional chemical extraction can substantially increase recovery by promoting biological transformation of the minerals the gold is trapped in [32,33]. The first bioleaching plant for copper in the United States was opened in 1989 [34].

Recent technical data on the operation (usage of water, energy, air, or chemicals) of bioleaching plants are rarely published. Usually, a 5–20% (w/w) ore suspension is used in reactors with capacities from 600 to 600 000 liters [10,26,31,34]. In processes independent of reactors ('heap leaching') 80 000 liters of water was used per minute in a sprinkler system to leach metals from 1.5×10^6 tons of low-grade (0.3%) copper ore [1]. The copper content of the leachate was 0.5 g/l. The obtained copper represented 30% of the total copper obtained by conventional winning techniques from this mine.

Several leaching processes of metals from ores have been recently patented. A summary is given in Table 3. Most of the patents are filed for the winning of precious metals such as silver or gold and chemolithoautotrophic microorganisms such as *Thiobacillus* are applied for metal mobilization.

4. Solid wastes as metal resource

Industrial waste materials (e.g., slag, galvanic sludge, filter dust, fly ash) often represent valuable metal-containing concentrates. For these materials biological processing can be economically effective and is thought to be an important industrial process to recycle elements (Table 4). Mostly *Thiobacillus* strains are applied for metal solubilization. However, also heterotrophic microorganisms such as *Aspergillus* are used to remove metal from solid waste (Table 4). These industrial wastes serve as solid substrates for bioleaching processes and can be considered a 'renewable resource' decreasing the demand for metal supply by mining activities. So far, leaching processes have been applied to industrial solid waste materials mainly for the recovery of copper and zinc. They are extracted with efficiencies of close to 100% [55].

In addition, the use of microorganisms is also feasible for detoxification applications to reduce environmental pollution. Metal-contaminated soils and sediments have been microbiologically treated using various *Thiobacillus* species [58–60]. It has been shown for lead-, zinc-, copper-, and arsenic-containing soil that leaching efficiencies depend on the content of reduced sulfur compounds, on the content of organic carbon, and on the buffer capacity. Also, climatic conditions have to be considered when soils are treated in situ. Usually, at temperatures $< 15^\circ\text{C}$ the leaching process not very effective [61].

5. Case study: Fly ash from municipal waste incineration

Slag and ash from municipal waste incineration

Table 3
Patents on biohydrometallurgical processing of ores

Type of ore	Metals recovered	Microorganisms	Reference
Iron ore	Fe	<i>Pseudomonas</i> sp.	[35]
Gold ore	Au	<i>Chromobacterium violaceum</i> , <i>Chlorella vulgaris</i>	[36]
Carbon containing gold ore	Au	<i>Thiobacillus</i> sp., heterotrophic fungal and bacterial strains	[37]
Sulfidic gold ore	Au, Ag, Cu	<i>Thiobacillus ferrooxidans</i>	[38]
Manganiferous silver ore	Ag	<i>Bacillus</i> sp., <i>Bacillus polymyxa</i>	[39]
Sulfidic ore	Au, Ag, Cu	<i>Thiobacillus</i> sp., <i>Leptospirillum ferrooxidans</i>	[40]
Sulfidic ore	Au, Ag, Pt	sulfate and hydrogen reducing bacteria	[41]

Table 4
 Industrials wastes as solid substrates for bioleaching processes

Type of waste	Metals recovered	Microorganisms	References
Sewage sludge	heavy metals	Thiobacilli	[22]
Galvanic sludge	Cu	Thiobacilli	[42,43]
	Cu, Cr, Zn	<i>Thiobacillus thiooxidans</i>	
Red mud from alkaline Al extraction	Al	Thiobacilli, <i>Aspergillus niger</i> , <i>Penicillium notatum</i> , <i>Penicillium simplicissimum</i> , <i>Trichoderma viride</i>	[23]
Filter press residues	Cu, Cr, V, Zn	<i>Thiobacillus thiooxidans</i>	[43]
Filter dust	Cu, Cr, Zn	<i>Thiobacillus thiooxidans</i>	[43]
Various filter dusts	Zn	<i>Thiobacillus thiooxidans</i>	[44]
Coal fly ash	Al	<i>Aspergillus niger</i>	[45,48]
	metals	<i>Thiobacillus thiooxidans</i>	[46]
	metal oxides	<i>Rhodococcus</i> sp.	[47]
Electro-filter dust from heavy oil combustion	Ni, V	<i>Thiobacillus ferrooxidans</i> , <i>Aspergillus niger</i> , <i>Penicillium simplicissimum</i>	[49]
Municipal solid waste fly ash	Zn	<i>Thiobacillus thiooxidans</i>	[43]
	heavy metals	<i>Aspergillus niger</i>	[5]
Oxidic fly ash from pyrite roasting	Cu	<i>Thiobacillus ferrooxidans</i> , <i>Thiobacillus thiooxidans</i>	[50]
Sulfidic dust concentrates after flotations	Cu, Zn	<i>Thiobacillus ferrooxidans</i>	[50]
		<i>Thiobacillus thiooxidans</i>	[44]
Filter dust of a copper works	Cu, Pb, Zn	<i>Brettanomyces lambicus</i>	[51]
	Zn	<i>Penicillium simplicissimum</i>	[52]
	Zn	<i>Pseudomonas putida</i>	[16]
Soda slag	Zn	<i>Thiobacillus thiooxidans</i>	[43]
Slag from lead melting process	Cu, Zn	<i>Thiobacillus ferrooxidans</i>	[50]
		<i>Thiobacillus thiooxidans</i>	[44]
Nickel matte (lead smelting)	Co	<i>Thiobacillus ferrooxidans</i>	[53]
Solid waste of a tanning factory	Cr	<i>Penicillium simplicissimum</i>	[11]
Used catalyst from silane production	Cu	<i>Thiobacillus ferrooxidans</i>	[54]
	Cu, Pb, Sn	<i>Bacillus</i> sp., <i>Saccharomyces cerevisiae</i> , <i>Yarrowia lipolytica</i>	[55]
Residue from zinc electrolysis	Zn	<i>Thiobacillus ferrooxidans</i> , <i>Thiobacillus thiooxidans</i>	[50]
Coprecipitated metals	metals	<i>Clostridium</i> sp.	[56]
Semi-conductors, electronic scrap	Ga, As	' <i>Sulfolobus</i> '	[57]
Electronic waste	Cu, Pb, Sn	<i>Bacillus</i> sp., <i>Saccharomyces cerevisiae</i> , <i>Yarrowia lipolytica</i>	[55]

represent concentrates of a wide variety of elements. These incineration residues can be considered 'artificial ores'. Some of the elements (i.e., Al, Zn) are present in concentrations that allow economic recovery of the metal (Table 1). Other compounds (i.e., Ag, Ni, Zr) show relatively low concentrations (comparable to low-grade ores), which makes conventional technical recovery difficult. Especially in these cases, microbial processes are the technique of choice and basically the only possibility to obtain metal from these materials. In addition, the low toxicity of these residues to bacteria has been demonstrated [62]. Therefore, a biological metal recovery process is potentially feasible. In a recent study, *Pseudomonas putida*, *Aspergillus niger*, *Thiobacillus thiooxidans* and *T. ferrooxidans* were used as test organisms and in-

cubated with fly ash obtained from municipal waste incineration.

When *P. putida* (DSM 548) was exposed to fly ash in batch cultures (baffled shake flasks, 110 rpm), citric acid – a product from the carbohydrate metabolism – acted with its complexing properties as leaching agent. The composition of the growth medium has been published elsewhere [63]. Glucose (24–42 g/l) was used as sole carbon source. Within a growth period of less than one week the pH dropped to about 4. Highest metal leaching efficiencies were in the range of 60–80% of the total content of a certain element present in the fly ash. For fly ash concentrations of > 30 g/l, efficiencies were below 20% for all elements (Fig. 1). The reason was a limitation of the carbon source. Therefore, the pH did

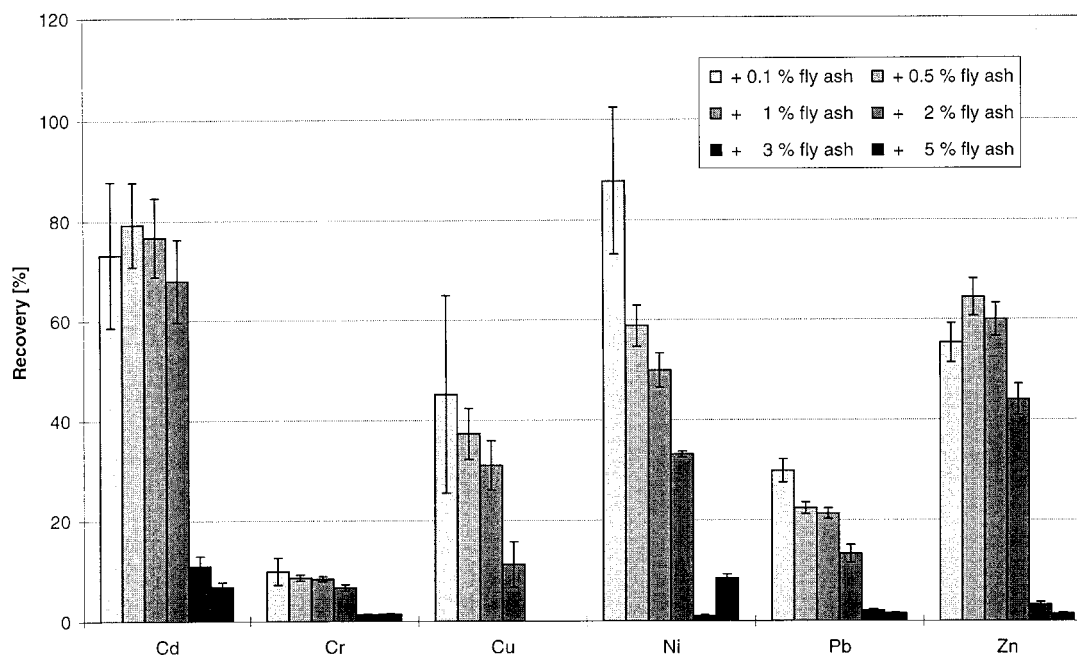


Fig. 1. Efficiency of heavy metal removal from various amounts (w/v) of municipal waste incineration fly ash by *Pseudomonas putida* when grown in batch cultures (baffled shake flasks, 110 rpm) on glucose for 5 days at 30°C. The pH decreased constantly during growth on 1% fly ash from 8 to approximately 4. Microbially produced citric acid acted as leaching agent. A detailed description of the chemical composition of the fly ash has been published elsewhere [4,5].

not decrease further and the ratio of citrate to fly ash was lower [64].

In the presence of fly ash, the heterotrophic fungus *A. niger* produced gluconic acid instead of citric acid due to the inhibition of enzymes in the TCA cycle by manganese. The chemical composition of the growth medium and the fly ash as well as culture conditions have been published elsewhere [5]. On sucrose (100 g/l), growth was completed after 2–3 weeks and the pH decreased within the growth period from approximately 10 to 4–5 depending on the amount of fly ash added. As a result, metal leaching efficiencies of >80% for certain elements were observed (Fig. 2). *A. niger* proved especially effective for the leaching of Pb [5].

Sulfuric acid produced by the autotrophic organism *T. thiooxidans* (DSM 504) during oxidation of elemental sulfur acted as leaching agent as well (Fig. 3). The growth medium consisted of tap water, 1% (w/v) elemental sulfur, and 4% (v/v) anoxic sewage sludge as inoculum to enrich Thiobacilli. In addition, a pure culture of *T. thiooxidans* was added after

3 days to the enrichments to enhance metal leaching. Cultivation was performed in batch cultures (baffled shake flasks, 110 rpm). Growth and pH decrease depended on the amount of fly ash added and generally lasted 1–3 months. At the end of the growth phase, the pH had dropped to a value of approximately 1. Elements such as cadmium, copper, or zinc were mobilized by >80%, whereas others (e.g., Pb) were solubilized only by a small percentage [64].

Biohydrometallurgical processing of fly ash poses serious problems especially at higher pulp densities, because of the high content of toxic metals and the saline and strongly alkaline (pH > 10) environment. For examining the biological leaching of fly ash at high pulp densities, pulp (80 g/l) from a storage vessel was mixed with a culture of *T. ferrooxidans* and *T. thiooxidans* in such a way that three stirred reaction vessels (vessels A, B, and C; each of them 1 dm³) ran serially in a semi-continuous process at pulp densities of 48 g/l [65]. The overflow of vessel A was fed to vessel B, and the overflow of vessel B was fed to

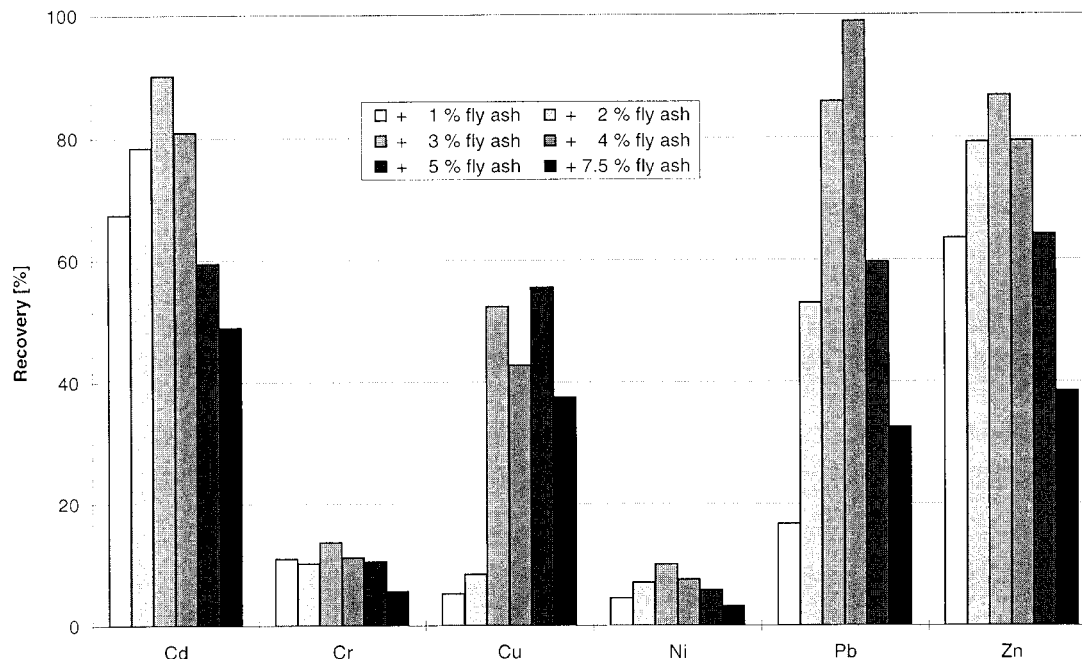


Fig. 2. Efficiency of heavy metal removal from various amounts (w/v) of municipal waste incineration fly ash by *Aspergillus niger* when grown in batch cultures (baffled shake flasks, 130 rpm) on sucrose at 30°C [5]. Microbially produced gluconic acid acted as leaching agent. Incubations were carried out until final pH was constant. Therefore, incubation times varied between 16 and 22 days depending on the amount of fly ash added. The chemical composition of the growth medium and the fly ash has been published elsewhere [5].

vessel C in order to decrease the pH stepwise from bioreactor to bioreactor. The results show that leaching efficiencies were of the same order as in experiments where *T. thiooxidans* was grown in batch cultures (Fig. 4). However, by employing a semi-continuous process higher pulp densities can be applied resulting in increased overall loads of elements in a shorter time period as compared to batch cultures. This experimental installation seems to be a promising first step on the way to a pilot plant with high capacities to detoxify fly ash and to recover metals from this material [65].

The results show the potential of different micro-organisms to leach substantial quantities of toxic metals from fly ash. Depending on the point of view, the mobilization or bioleaching of these metals could be either a hazard for the environment (leachates from landfills or ore deposits) or a chance to reduce toxic elements (to fulfil the regulations for landfilling or even better for a re-use of the fly ash, e.g., for construction purposes) and to recover valuable metals (e.g., zinc) by a low cost and low energy

level technology compared with thermal treatment (e.g., vitrification or evaporation).

6. Perspectives for bioleaching processes

According to the Agenda 21 established at the Earth Summit in Rio de Janeiro (1992), it is a strong requirement that in the field of biotechnology progress must be made towards a more environmentally friendly winning of mineral resources [66]. The application of biological metal leaching processes is a step in this direction. Bioleaching technologies described must be seen in the context of a future in which industrial technologies have to be more in harmony with global material cycles within the biosphere [67]. There are several advantages of bioleaching techniques compared to chemical leaching (Table 5).

Industrial technologies of the future must be friendly to humans and the environment. They are based on four key characteristics, namely (i) they

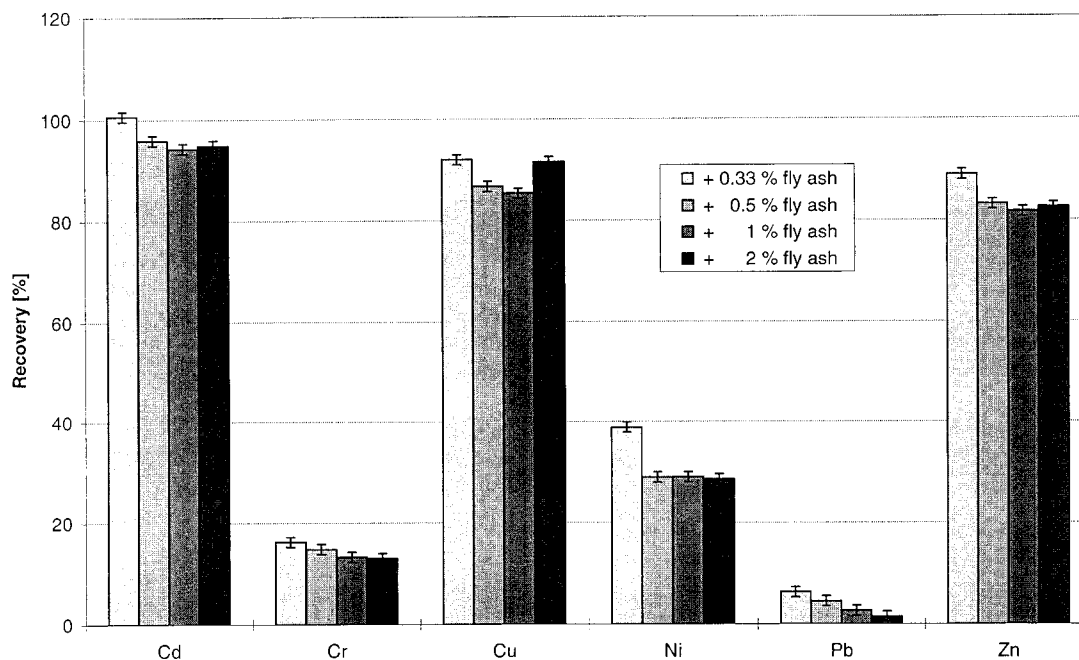


Fig. 3. Efficiency of heavy metal removal from various amounts (w/v) of municipal waste incineration fly ash by an enrichment of *Thiobacilli* from anoxic sewage sludge. Growth was performed chemolithoautotrophically in batch cultures (baffled shake flasks, 110 rpm) for approximately 60 days at 30°C. The chemical composition of the fly ash has been published elsewhere [5]. Microbially produced sulfuric acid acted as leaching agent. pH decreased during the growth period from approximately 10 to about 1.

should be based on renewable resources, (ii) they should use 'mild' production processes, (iii) resulting products and services should be environmentally compatible, and (iv) any waste they generate should be recyclable [66]. Whereas classical bioprocesses have been applied for decades (i.e., food processing) often on a large scale, process-integrated industrial biological technology ('clean technology') is still in its infancy. Industry views this as a very promising technology for sustainable development [67]. Espe-

cially metal recovery by biological means should be improved.

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Table 5

Biological leaching of metals from waste incineration residues compared with thermal treatment or chemical leaching

Advantages	Disadvantages
<ul style="list-style-type: none"> – leaching agents are produced in situ (no need for transportation) – formation of a micro-climate around particles with elevated concentrations of leaching agents – microbial selectivity depending on strain used and leaching conditions – increase in leaching efficiency – excretion of surfactants – low energy demand – no emission of gaseous pollutants 	<ul style="list-style-type: none"> – long reaction times – dependence on climate (when residues are treated in the field) – heavy metal toxicity to microorganisms – potential for acid leaks

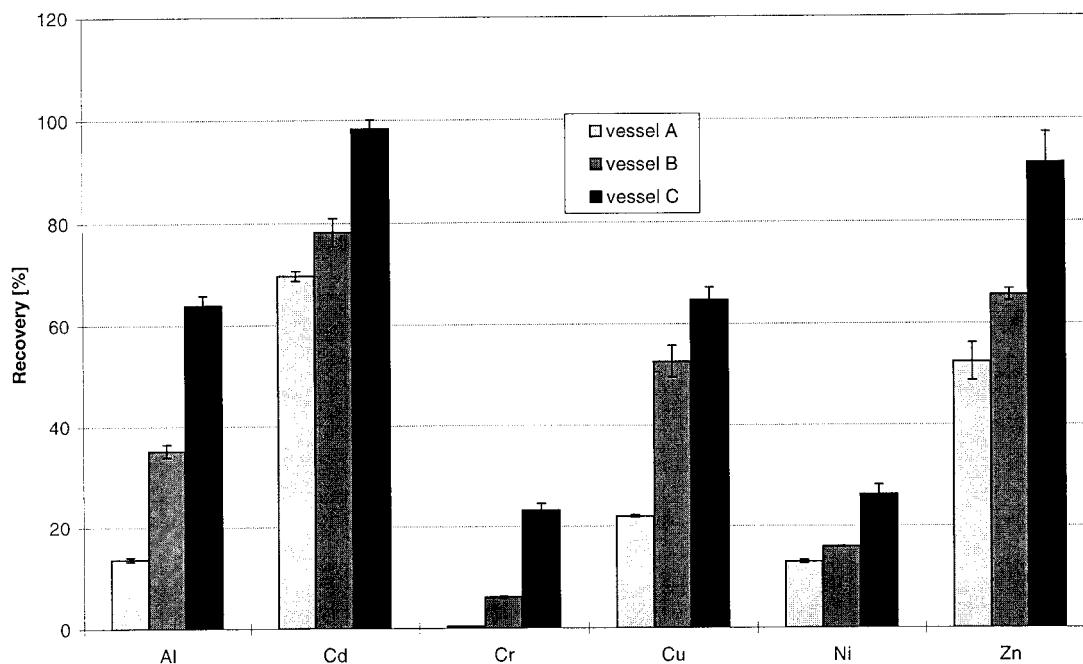


Fig. 4. Efficiency of heavy metal removal from various amounts (w/v) of municipal waste incineration fly ash by *Thiobacillus thiooxidans* and *T. ferrooxidans* at pulp densities of 4% (w/v) [65]. Metal leaching was performed semi-continuously at room temperature (22°C) in a series of three connected reaction vessels (A, B, C) with retention times of 2 days in each vessel. pH values in vessel A, B and C were approximately 3, 2 and 1.5, respectively, and were constant throughout the experiment. The growth medium consisted of (grams per liter of distilled water) K_2HPO_4 (0.1), $MgSO_4 \cdot 7H_2O$ (0.25), $(NH_4)_2SO_4$ (2.0), KCl (0.1), $FeSO_4 \cdot 7H_2O$ (8), powdered sulfur (10) and H_2SO_4 (1 ml). pH was 2.5–2.7.

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